

The Effects on Zebrafish Embryos After 5 Days of Being Exposed to the Nicotine

Kaylee Builer

Waukesha North High School

Abstract

Research has shown that nicotine affects developing embryos by slowing developmental rate and even killing them. It was important to know this to understand the impacts of embryonic development so the zebrafish were being accurately observed. The purpose of the experiment was to see how nicotine affects developing zebrafish, and how that relates to humans when a mother smokes while pregnant. During the experiment, the first step was to equally separate 40 zebrafish into four categories, all of which were exposed to increasing concentrations of nicotine. Then, the zebrafish were observed over five days. It was found the the zebrafish in higher concentrations developed slower, took longer to hatch, and had a higher mortality rate. Meaning the nicotine negatively affected them compared to the control. This relates to humans because, zebrafish react similar to the way humans do. This means if a mother smoked while pregnant their child may react similarly to the zebrafish. So, if the zebrafish developed slower then it is possible humans will as well, and because the zebrafish had a higher mortality rate, infants could develop sudden death syndrome.

Introduction

How have children been affected if their mother smoked while pregnant with them? This was the main question that the experiment was based on. According to NIDA, (2018) “Smoking while

pregnant may also be associated with learning and behavioral problems in exposed children.”

That meant that when a woman smokes then children will develop behavioral problems. This is important because if children whose parents smoked while pregnant with them, could be at risk for more serious issues. While observing the zebrafish we will be able to see if this is true for the zebrafish as well. According to Tara Zubbarao (2006), “Parental exposure to nicotine has been correlated with a number of abnormalities including spontaneous abortions, Sudden Infant Death Syndrome, and low birth weight, as well as behavioral, cognitive, and intellectual impairment” (p. 4). That meant that if a woman smokes while pregnant, then their child will could experience things like Sudden Death Syndrome, as well as other issues. SDS is something we can test on our zebrafish embryos, by seeing if the zebrafish die after exposure to nicotine. They also lack spontaneous muscle bends when they are exposed to nicotine. According to Kurt Svoboda (Accessed 2018), 13-17% of women smoke and it is affecting the health of children. What that meant was, a baby whose parents smoked while pregnant with the would be weaker. The paper also explained that parents that smoke while pregnant have a infant mortality. The article proved smoking while pregnant is both bad for the parents, and very bad for the child. According to Andrew M. Petzold (November 3 2009), zebrafish have many of the same parts to their brain as humans and are a good way to experiment. That meant zebrafish are similar to humans in how they would react to nicotine. Because of this, zebrafish embryos were a good way test the effects of nicotine. Therefore the experimental hypothesis was, if the zebrafish embryos are exposed to nicotine then they will hatch slower and have a higher mortality rate because nicotine can cause sudden infant death syndrome.

Materials and Methods

Materials

- 40 Zebrafish Embryos
- Stock Solutions of Nicotine, 0.0 mg/mL (control), 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL.
- 1 Multi-well Plate
- 1 100 mL Beaker for Dead Embryos and Liquid Disposal
- 1 Sharpie
- 1 Transfer Pipettes
- 5 Fine Bore Pipettes
- 1 28.5 degrees Celsius Incubator
- 1 Dissecting Microscope
- 1 Procedure Guide

Method

Four groups of ten healthy zebrafish embryos were placed into a multi-well plate, in four different wells. Then, the residual water was removed from each well, with a fine bore pipette. The water, with four different concentrations of nicotine, was placed into the four wells one milliliter of the four concentrations. Gloves were worn as a precaution, when transferring and working with chemicals. In addition, washing hands after transferring nicotine is a very important procedure, as nicotine is toxic. The concentration levels were 0.0 mg/mL (control),

0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL. The zebrafish embryos were placed into a incubator at 28.5 degrees celsius overnight.

After 24 hours, the zebrafish were observed. Over the course of four days, data was collected on the zebrafish. To do this, the zebrafish embryos were placed under a dissecting microscope. Data was recorded on how many had hatched, and how many had died. If the zebrafish were very black under the microscope, it would indicate that the fish were dead. If any had died, the fine bore pipette was used to remove the waste. In addition, a microscope was used to find the heart rate of the zebrafish. This was found by looking for the heart, located just above the egg sack, and then counting the amount of times the heart beat in 10 seconds. The fine bore pipette was also used to remove the nicotine from each well, and replace the old nicotine concentration with new concentrations of nicotine. Each day, care was taken to put the in 1 mL of solution 0.0 mg/mL (control group), 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL of concentration in each well.

On day five, or the last day, the same previous steps were first taken. However, what made this day different, was at the end all zebrafish embryos, except the control group, were disposed of in a waste container. The fish still alive, and that had hatched, within the control group were placed in a large aquarium, with other groups healthy zebrafish. This allowed the teacher to try and take care of the remaining zebrafish.

A Chi square analysis of the data was completed to ensure statistical significance

Results

The purpose of having done this research was to find how nicotine affects zebrafish, and how it potentially can affect a developing human fetus. This was stated in the experimental

hypothesis that stated, if the zebrafish embryos are exposed to nicotine then they will hatch slower and have a higher mortality rate because nicotine can cause sudden infant death syndrome. The experiment was set up based on previous research and instructions. The dependent variable of the experiment was the amount of fish that died and how fast the fish hatched. Then, the independent variable was the nicotine the zebrafish were exposed to which were 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL. The control group was the zebrafish that were exposed to the 0.0 mg/mL of nicotine, and the controlled variables were the amount of solution and incubation temperature. The independent and dependent variables were connected because as the concentrations of nicotine increased, there was a higher mortality rate and a slower hatch rate. To assess significance, a chi square analysis was conducted. The degree of freedom was 3, the critical value was 7.82. The Chi Square value was 17.62, which means that the null hypothesis was rejected. The null hypothesis rejection meant the zebrafish did, in fact, have a negative reaction to the nicotine. This information directly correlates to table one. Table one showed that the higher concentrations of nicotine did indeed have a higher mortality rate than the fish embryos in the control group and the low concentration.

The Number of Live Zebrafish Over the Course of Five Days After Being Exposed to Nicotine

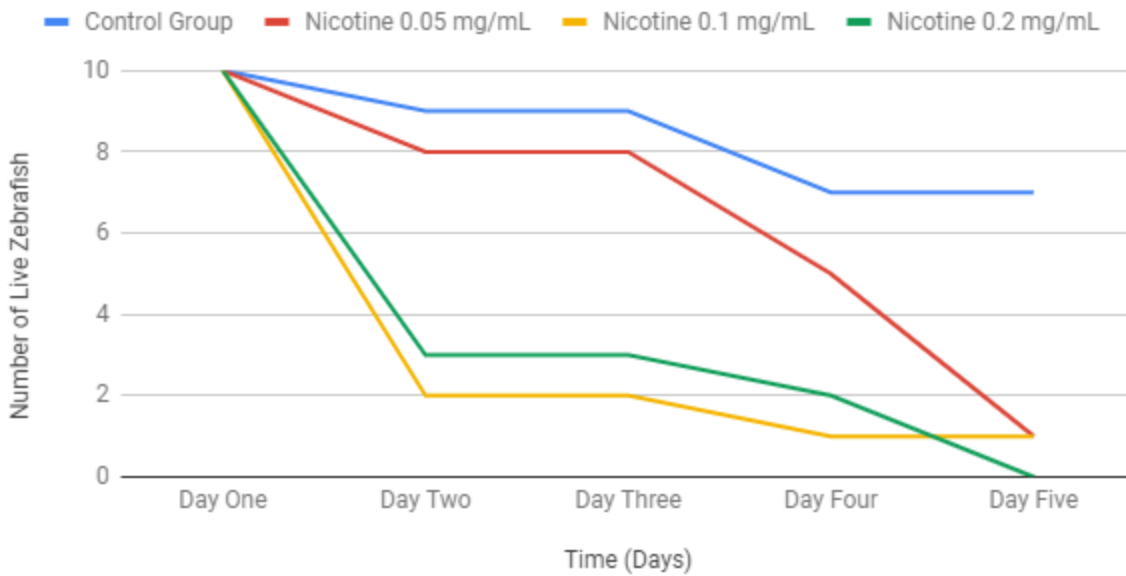


Figure one shows that the experiment started with ten fish in each nicotine concentration. At the end of the five days the control group had seven living zebrafish, the 0.05 mg/mL nicotine concentration had one living zebrafish, the 0.1 mg/mL nicotine concentration had one living zebrafish, and the 0.2 mg/mL nicotine concentration had no living zebrafish.

The Number of Living Hatched Zebrafish Over the Course of Five Days After Being Exposed to Nicotine

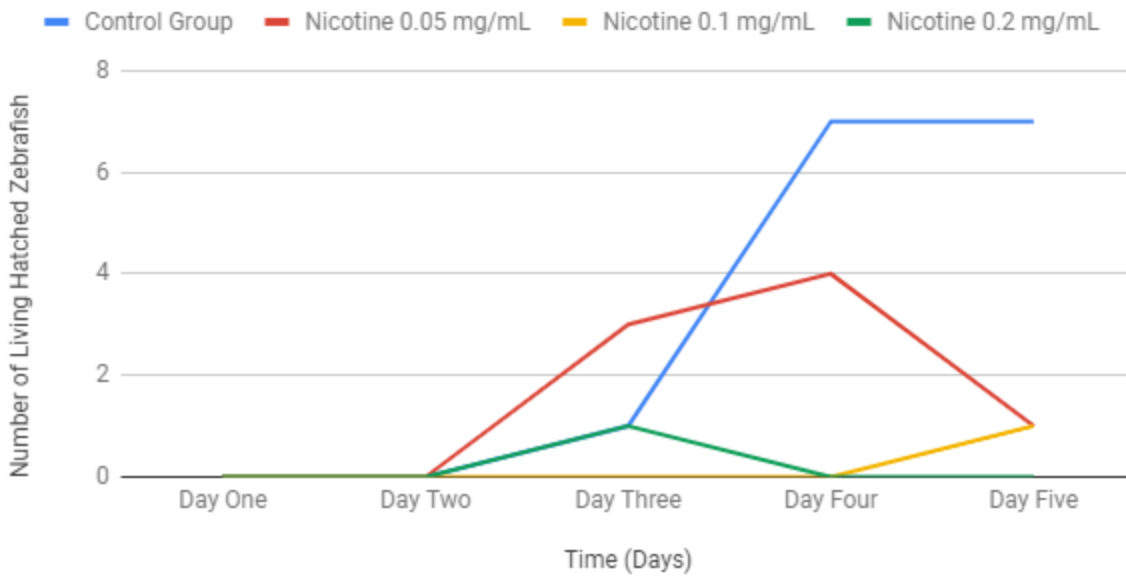


Figure two shows that there were no living hatched zebrafish until day three. At the the end the control group had seven living hatched zebrafish, the 0.05 mg/mL nicotine concentration had one living hatched zebrafish, the 0.1 mg/mL nicotine concentration had one living hatched zebrafish, the 0.2 mg/mL nicotine concentration had no living hatched zebrafish.

Treatment	#of starting fish	24 hours		48 hours		72 hours		96 hours	
		# hatched	# live	# hatched	# live	# hatched	# live	# hatched	# live
Control Group	10	0	9	1	9	7	7	7	7

0.05 nicotine mg/mL	10	0	8	3	8	4	5	1	1
0.1 nicotine mg/mL	10	0	2	0	2	0	1	1	1
0.2 nicotine mg/mL	10	0	3	2	3	0	2	0	0

Table one above, shows the amounts of nicotine in the different groups of zebrafish. This also shows the amount of hatched and alive fish after each time period post fertilization.

Discussion

Previous research showed, that if a mother had smoked while pregnant, their child was more likely to develop mental deformities, slower development/stunted growth, and even death. Those traits were things that could be studied in zebrafish because zebrafish react similar to humans. This is because they have similar parts of the body, such as, heart and brain which means they give very accurate results. When conducting the research, it was found that zebrafish in higher concentrations of nicotine, had a higher mortality rate, and a slower hatch rate. The more time that went on, the more embryos in higher concentrations of nicotine died. This proved that nicotine did affect zebrafish, in a negative way. The way they reacted was negative because the zebrafish were getting sick and started dying when there was too much nicotine in their well

as well as having slower development rate. If zebrafish reacted similar to people, they would also be negatively affected, such as dying, slowly developing, and getting sick. This proves the experimental hypothesis was correct because, the zebrafish in higher concentrations of nicotine, had a higher mortality rate and a slower hatch rate.

Some limitation that were in this project included us only having one test trial, small sample sizes, different test times, short test time, and lacking superior materials like larger wells, better transporting tools, etc. To improve this experiment, we could have had more time, more trials, larger sample sizes, and more zebrafish embryos. The main error that happened was the zebrafish not being observed over consistent time periods. This is because we weren't always able to come into class at the same time, which could have an effect on our data. The data from the experiment proved that the claim was correct which was, if the zebrafish embryos are exposed to nicotine then they will hatch slower and have a higher mortality rate because nicotine can cause sudden infant death syndrome and childhood learning disabilities. However, one question that remained unanswered was, what would have been the outcome of the fish if we would have raised them longer in the different concentrations?

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